consisting of a phospholipid (dioleoylphosphatidylcholine), cholesterol and diacylglycerol in a 50:50:3 mol ratio. In this protein-free system, fusion occurs just by thermal fluctuations, above 60°C. Under our conditions cholesterol is essential to produce vesicle aggregation, but fusion is only observed when small amounts of diacylglycerol are added. Vesicle fusion occurs only under conditions when X-ray diffraction and cryo-transmission electron microscopy of the lipid mixtures used in vesicle preparation show inverted lipid phase formation (hexagonal and cubic).

3075-Plat
DNA-Mediated Fusion Between Small Vesicles and a Planar, Tethered Bilayer Patch
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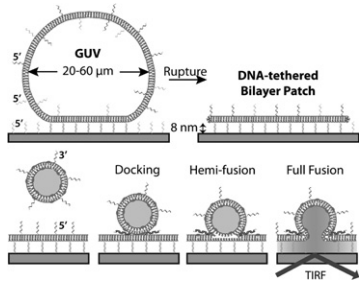
We have previously shown that DNA-lipid conjugates can be used to mediate membrane fusion between small vesicles and also to construct membrane architectures, such as a DNA-tethered lipid bilayer patch which is distanced from a glass coverslip by 8 nm long DNA duplex tethers. We currently employ these bilayer patches in a model vesicle fusion system, studying the fusion (both lipid-mixing and content-mixing) of small vesicles to the bilayer patches, where fusion is mediated by complementary DNA-lipid partners in a geometry similar to that of SNARE-mediated membrane fusion. This model system allows us to quantitatively describe important aspects of membrane fusion, such as the dependence of fusion on DNA-lipid concentration, the numbers of DNA-duplexes involved in the fusion process, the role of proximity and membrane curvature on the fusion reaction. We have also developed a kinetic model describing the distribution of docking-to-fusion wait times and find that these wait times are exponentially distributed, implying a stochastic process which initiates fusion. Insights into the biological membrane fusion process are discussed.



3076-Plat
Watching Influenza get Hogtied
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Influenza viruses have hundreds of hemagglutinin (HA) protein trimers embedded in their phospholipid envelope. The HA protein is the lynchpin in the first stage of influenza infection, first by facilitating binding and internalization into target host cells and then by mediating fusion with the membrane of late endosomes. Recently, antibodies capable of neutralizing both group 1 and group 2 influenza A viruses have been described [Science,v324,p246,2009; Science,v333,p843,2011]. These broadly neutralizing HA stem-binding antibodies appear to act by blocking the HA conformational change necessary for fusion. Here, we have visualized the interplay between a virus and antibodies during neutralization using our recently developed single-particle fusion assay [PNAS,v105(40),p15382,2008]. To this end, antibodies and infectious viral particles were fluorescently labeled and incubated together. Viruses were then bound to sialic acid decorated proteins incorporated into a planar, supported phospholipid bilayer and fusion induced by addition of an acidic buffer. Using TIRF microscopy, individual hemifusion events were observed as the rapid release of lipophilic dye from the viral membrane into the target bilayer. This novel approach allows us to directly link the action of individual HA-binding antibodies to viral fusion events, providing new insight into the mechanism and molecular stoichiometry involved in fusion and neutralization - information obfuscated in traditional cell-cell and bulk fusion assays. We see a concentration dependent effect of inhibiting antibodies on the time it takes for viruses to undergo hemifusion as well as on the total number of hemifusion events. Furthermore, we can correlate the fluorescence intensity per virion to the number of bound antibodies and thereby directly measure the number of HA molecules involved in fusion. More to the point, our results have explicit implications for the model of bilayer fusion arising from the coordinated efforts of multiple hemagglutinins.

3077-Plat
A Microfluidic Device for Single Cell-Liposome Fusion
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The fusion of lipid membranes is ubiquitous in living organisms and allows the transfer of membrane-impermeable molecules across the cell membrane and different cell compartments. With the aim to systematically investigate membrane fusion, we developed an advanced microfluidic device that facilitates the close contact positioning of single cells and small unilamellar vesicles (SUVs) and enables chemically induced fusion. The SUVs were immobilized on discrete, micro contact printed spots on a glass surface. For vesicle immobilization, we used biotin-PEG-cholesterol bound to immobilized avidin. Supply of the SUVs at low flow rates and incubation times of a few minutes enabled efficient immobilization of the SUVs on the designated areas due to the insertion of cholesterol into the lipid membrane. The patterned glass was covered by a microfluidic device made of poly(dimethylsiloxane)(PDMS) that contained the micro-channels for fluid supply and an integrated array of one hundred geometric hurdles to trap individual cells. We studied the fusion of cells that are trapped just above the immobilized SUVs by using total internal reflection fluorescence microscopy and wide field fluorescence microscopy. In preliminary experiments, the pH-induced fusions of suspension cells (U937) to SUVs prepared from anionic and fusogenic phospholipids (DOPG and DOPE) were investigated. A fluorescence dequenching assay with two fluorophores (R18 and HPTS) was employed to visualize, in real time, the fusion of cell and liposome membranes and the release of the liposome's enclosed fluids into the cytosol. The novel platform will expand our knowledge of essential cellular processes. Moreover, it facilitates screening of optimal fusion parameters and hence, could improve the typically low efficiency of cell lipofection to deliver DNA and other biomolecules into the cytosol. This would ultimately lead to novel therapeutic strategies, e.g. against cancer.

Platform: Membrane Transporters & Exchangers II

3078-Plat
Towards a Structural Understanding of Alternating Access within the Proton Dependent Oligopeptide Transporter (POT) Family
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Recently we determined the first crystal structure of a member of the Proton dependent Oligopeptide Transporter (POT) family, PepTSo, a prokaryotic homologue of the mammalian PepT1 and PepT2 proteins. PepT1 and PepT2 are responsible for the uptake and re-absorption of dietary peptides and have a direct effect on the oral bioavailability of important drug families. POT family transporters belong to the Major Facilitator Superfamily (MFS) of secondary active transport proteins and contain between 12 and 14 transmembrane helices. The structure of PepTSo revealed a ligand bound occluded state that was noticeably asymmetric between the two canonical N- and C-terminal six helical bundles present in MFS members. To gain further insight into the transport mechanism of the POT family we have determined the structure of a second member to 3.3 Angstrom resolution. Compared to PepTSo, this second structure reveals an essentially symmetric inward open conformation. Here we present the analysis of this structure and show that using previously identified inverted topology repeats present within the MFS fold we can create a model for the currently missing outward open conformation. We further provide experimental evidence for the outward open model using double electron electron resonance (DEER) measurements. These two crystal structures, combined with the repeat swapped model, essentially describe the basic set of conformations required for the alternating access model of transport within this pharmaceutically important family of MFS transporters.

3079-Plat
Conformational Transition Pathway of GlpT Transporter, Characterized by Nonequilibrium Molecular Dynamics Simulations
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Glycerol-3-phosphate transporter (GlpT) is a member of major facilitator superfamily (MFS), the largest family of secondary transporters. Similar to other transporters, GlpT is believed to function using an ''alternating-access'' mechanism during which it undergoes large conformational changes. The only available structure of GlpT is in the *apo* inward-facing (IF) state. In our earlier work using equilibrium molecular dynamics (MD) simulations, the binding site of GlpT was characterized and the initial substrate-induced closing on